Summary

Pinus Douglasiana was perhaps included by Shaw under *P. pseudostrobus* var. *tenuifolia* (Benth.) Shaw, but it differs from this in its longer, stouter, leaves and larger apophyses of the cone scale. It occurs from Sinaloa to Oaxaca. It is named in honor of Mrs. Margaret Douglas.

Morelia 61, México, D. F.
Mayo de 1942.

AN INVESTIGATION OF THE PRESENCE OF SILICEOUS RODS IN THE SECONDARY WALL OF WOODY TISSUE[1]

Walter M. Schall

In 1920, Forrest B. H. Brown (3) proposed an explanation of differential wood shrinkage by stating that a skeleton of siliceous rods existed within the secondary wall of wood elements. He assumed that these rods, acting as a restraining framework, kept longitudinal shrinkage at a minimum. Since the presentation of this explanation, many workers in cell wall structure (6, 7, 12) have referred to this siliceous skeleton or have tacitly assumed its presence, notwithstanding the fact that the micelle theory advanced by Nägeli (13) in 1863 and substantiated by subsequent workers (1, 2, 7, 11, 12, 17) is now generally accepted as the logical explanation for the shrinkage behavior of wood. The present study was undertaken not to explain the mechanics of shrinkage but rather to investigate the procedure employed by Brown (3), first to check his results and second, if similar results could be obtained, to interpret them in the light of the accepted theories regarding wood shrinkage.

The author wishes to give acknowledgment and thanks to Dr. R. A. Cockrell for his interest and suggestions, both in conducting the investigation and writing the final manuscript.

In repeating Brown's (3) work, the following species of wood were used: *Swietenia mahogoni, Trochodendron aralioides, Quercus alba, Cedrela* sp., *Pinus strobus,* and a lapachol-forming species of *Tecoma. Tecoma,* according to Record (15, p. 532), is divided into four groups, "Prima vera," "roble," "ipé peroba," and "lapacho" or "páo d'arco." The "lapacho" group is characterized by having wood that is very "hard and heavy, has an oily olive-brown color, and the vessels are more or less completely filled with yellow crystalline substance (lapachol), which may give the surface the appearance of having been dusted over with sulfur. Ripple marks are always present and usually regular." Lapachol ($C_{15}H_{14}O_3$) "when moistened with ammonia or dilute

[1] Contribution of the Department of Forestry, University of California, Berkeley.

sodium carbonate, turns a deep wine-red, thus providing a reliable diagnostic feature." In order to give comparable results with Brown (3), the results of tests made on *Tecoma* sp. are completely reported although the same tests with comparable results were made on the other material.

If a siliceous skeleton were present in the secondary wall of woody tissue, a change in dimension might take place upon desilicification. To test this, following Brown's (3) procedure, blocks of the foregoing species were placed in hydrofluoric acid to dissolve silica thereby eliminating its reputed restraining effect on shrinkage. In addition, duplicate control blocks were placed in hydrochloric acid which does not dissolve silica but which in other respects should have an effect on the cell wall substance similar to that of hydrofluoric acid. The blocks, ranging in size from one-half to one inch in linear dimension, with true radial, tangential, and longitudinal faces, were measured with a micrometer and placed in boiling water. After five and one-half hours of boiling, during which the blocks were measured at one-half hour intervals, duplicate blocks of each species were immersed in separate solutions of concentrated hydrochloric and 52 per cent hydrofluoric acid. Measurements of the change in dimension were made after eighteen, twenty-three, twenty-eight and thirty-one hours' immersion. After washing in running water for ten hours, and measuring again, the blocks were placed in an oven at 103° C. to remove the excess moisture. The moist blocks were then allowed to air-dry for eight days. Since the swelling values for each of the species followed the same general trend, only the values for *Tecoma* sp. are included. These are recorded in Table I.

In order to supplement the block measurements, individual fibers obtained by maceration with concentrated nitric acid and potassium chlorate (4) were treated with both hydrofluoric and hydrochloric acid. The fibers were mounted in alcohol and measured both in length and width under the microscope. The alcohol was then allowed to evaporate and the acid was added. In no case did the fiber length change after contact with either acid for an hour. If silica in the form of rods was present, the time in hydrofluoric acid was sufficient to dissolve the silica from the fiber. Since no measurable change was observed, it must be concluded that the acids did not remove the restraining force holding the fiber cell wall together, and the cell wall must have been fully swollen before the acids were added.

The effect of chemical treatment on the physical characteristics of the wood was marked. In several cases the blocks showed a decided tendency to check and some even showed some collapse. These same blocks were brittle and a slight pressure on any of the faces caused splitting that took place tangentially along the annual rings as well as radially along the rays. It is not likely that merely drying the wood would cause such extreme stresses to

TABLE I

Percentage Swelling for *Tecoma* sp. in Water and Acid*

	Time	Tangential		Radial		Longitudinal	
Per cent change in boiling water	½ hour	5.4	7.0†	3.6	6.0†	0.8	0.1†
	1 hour	6.0	11.0†	3.9	10.0†	0.8	0.1†
	1½ hours	6.3	12.7†	3.9	11.0†	0.8	0.1†
	2½ hours	6.7	12.7†	4.2	11.0†	0.8	0.1†
	3½ hours	7.0		4.2		1.1	
	4½ hours	7.0	13.0†	4.2	11.0†	1.1	0.1†
	5½ hours	7.1		4.2		1.1	
Per cent change in concentrated hydrochloric acid at room temperature	18½ hours	12.2		7.3		0.3	
	23 hours	12.8		7.6		0.3	
	28 hours	12.8		7.5		0.3	
	31 hours	12.8		7.3		0.3	
Per cent change at air dry moisture content	8 days	−4.7		−4.3		0.1	
Per cent change in 52 per cent hydrofluoric acid at room temperature	16 hours	8.2	15.0†	4.4	11.0†	0.5	0.1†
	20½ hours	7.7	85.0†	4.6	43.0†	0.5	−18.0†
	27½ hours	7.5	57.0†	4.4	29.0†	0.3	−22.0†
	30½ hours	7.5		4.3		0.4	
Per cent change at air dry moisture content	8 days	0.1	−13.8†	2.2	−19.0†	0.0	−40.0†

* Values represent averages for at least four determinations. Negative values represent shrinkage.

† Results as obtained by Brown (3) for similar treatment.

be set up but it is probable that the splitting action was caused by a material weakening of the cell wall by chemical action. These checked blocks were disregarded and only apparently sound blocks were used for measurement.

In determining the air-dry moisture content before and after acid treatment, it was found that the hygroscopicity had decreased. After treatment with hydrochloric acid, the moisture content of the blocks was reduced to three-fourths of the original value and after treatment with hydrofluoric acid to one-third. Again, this would indicate that the acids caused a chemical and physical change in the minute structure of the cell wall so that its original equilibrium moisture content was significantly reduced.

Each of the untreated blocks was sectioned on a microtome, using a jet of a steam to soften the woody tissue. The sections, mounted in water, were examined at 1300 diameters under a Zeiss binocular microscope equipped with an oil immersion apochromatic objective (N.A. 1.3) and 10× compensating eyepieces. Likewise, sections mounted in glycerine were examined and even after contact with the liquid for twelve hours, in no case was there any indication that small isolated areas, supposedly the cut ends

of siliceous rods, were present. The photomicrograph of a cross section of *Tecoma* sp. (fig. 1) taken at 810 diameters shows no discontinuities in the cell wall.

The index of refraction of the cell wall, as found by Brown (3), was verified using McLean's Solution (4) of known indices on cross sections of *Tecoma* sp. Since the index of refraction of the secondary wall and silica are practically the same, the proximity of the two indices may have obscured any possible difference due to the presence of silica.

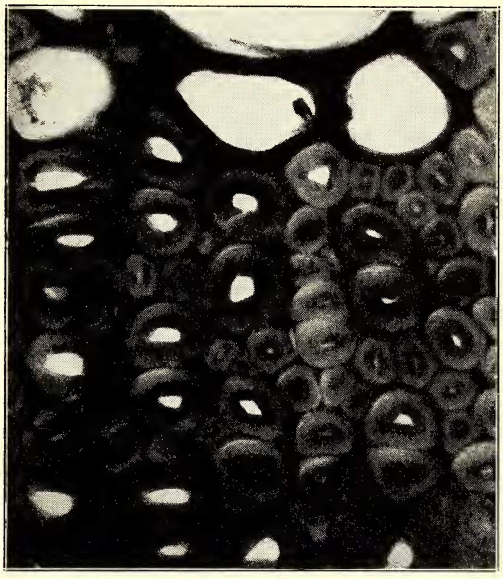

Fig. 1. Transverse section of a lapachol-forming species of *Tecoma*. ×810.

Both the cross sections and the individual fibers of *Tecoma* sp. were incinerated to see if visual evidence of the rods could be found during the course of incineration or in the ash. Individual cells were isolated from a section 14 microns thick and incinerated over an alcohol flame. The heating time was lengthened for successive sections so that examination could be made at varying degrees of incineration. Under the microscope, the ash showed irregularities but no regular arrangement of bodies was noted. According to Uber (19), these irregularities are probably a result of a natural tendency of the ash to check upon shrinkage. Macerated fibers were incinerated the same way but again no visual evidence of the localization of silica was obtained.

In addition to determining the effect of the presence of a siliceous skeleton on the shrinking and swelling of wood, the actual amount of silica present was determined. Following Brown's (3) procedure, this determination was carried out in two steps. The first was to determine the ash content and the second to determine the per cent silica in the ash. An oven-dry sample of *Tecoma* sp. was accurately weighed and ashed in an electric muffle at moderate red heat. The weight of ash thus obtained was 0.25 per cent of the dry weight of wood. A silica determination based on the weight of ash was made using hydrochloric and perchloric acid in the quantitative analysis (9). The weight of

silica thus obtained gave an average of 0.85 per cent of the dry weight of ash.

The values obtained for ash and silica content are considerably below those given by Brown (3). He states that, "after combustion, 1.8 per cent (of the dry weight of the fiber) of mineral matter was obtained and 0.1 per cent (of the dry weight of the fiber) of silica or silicic acid." These percentages are of little value since they are based on the dry weight of fiber which can be obtained only arbitrarily. The maceration procedure is extremely variable since neither the time nor the temperature of reaction is standardized. A trial maceration using concentrated nitric acid and potassium chlorate (15), gave a dry weight of fibers for *Tecoma* sp. equal to 42 per cent of the dry weight of wood. Based on the dry weight of fibers, this would give values for ash and silica content approximately twice as large as results based on the dry weight of wood if the assumption was made that all the mineral matter was retained by the macerated material. A comparison of ash and silica content cannot be made with any marked degree of accuracy, however, since within a single tree, the values vary from periphery to pith and from the base to the top of the tree.

It is not outside the realm of possibility that inorganic materials are centralized within certain areas in the cell wall. Kerr and Bailey (11, p. 285) state that "the central layer of normal tracheids, fiber-tracheids, and libriform fibers is composed, in all cases, of a complex and firmly coherent matrix of cellulose with elongated, intercommunicating interstices. Within these interstices more or less 'lignin' and other non-cellulosic constituents may be deposited." Bailey (1) recently states specifically that minerals may be deposited within these interstices. Since the mineral content occupies such a small percentage of the weight of materials within the interstices, it is improbable that silica would be so localized as to form a continuous rod.

In view of these findings, it is not likely that a highly silicified skeleton is present in the secondary wall of woody tissue. The results of swelling tests alone should be conclusive evidence of this fact since several species of wood were used and the blocks treated with two mineral acids, one of which would dissolve silica while each should have similar effects on the cell wall structure. If siliceous rods were present they would certainly have been disintegrated after immersion in 52 per cent hydrofluoric acid for 30 hours. As indicated in Table I, a small increase in size was observed but no appreciable difference was noted between the blocks placed in hydrofluoric acid and those placed in hydrochloric acid. Furthermore, the individual fibers failed to give a measurable change when each of the acids was added. In a schematic drawing of a fiber before and after hydrofluoric acid treatment, Brown (3) indicates that the diameter increases and the length decreases with the acid treatment. He explains the

change by stating that the siliceous rods had been broken, thus freeing the so-called homogeneous substance which could then swell without hindrance. If the cell wall is a homogeneous substance, the fiber should have increased in length and width in the same proportion and an inverse relationship could not have resulted.

The acids probably caused a degradation of both lignin and cellulose as well as attacking any of the minerals in the wood. This is borne out by the mechanical weakening of the wood and by the reduced hygroscopicity. Chamberlain (5) and Sacc (17) state that the action of hydrofluoric acid is to soften wood but they do not point out the manner of softening. Plowman (14) in 1904 stated that the action of hydrofluoric acid was to remove silica and other mineral deposits from the wood. Kerr (10) and Harlow (8) point out that the action of hydrofluoric acid is to attack the cell wall substance and the removal of silica is a secondary operation. Harlow (8) explains the softening action as a degradation of lignin since the treated wood does not respond to the Maulë reaction which is employed as a test for the presence of lignin. Kerr (10), on the other hand, states that the action is due to a degradation of cellulose to hydrocellulose and explains the action by drying other acids into wood in order to soften the woody tissue. Rudiger (16) points out that swelling precedes the dissolution of cellulose in liquid hydrofluoric acid and that the lignin structure of the membranes was destroyed, although the lignin itself did not swell. Forsaith (6) in his explanation of differential swelling of wood with adsorption of water credits the siliceous skeleton as proposed by Brown (3) as exerting an influence in conjunction with the micelle theory as proposed by Nägeli (13). A more recent worker, Maby (12, p. 434), in his explanation of shrinkage and swelling, says, "On the other hand, the longitudinal siliceous strands in the cell wall, noted and described by F. Brown, might be expected to exert a binding effect over dimensional changes in the longitudinal direction."

Schorger (18, p. 9) states, "Nägeli, as a result of his study of the growth of starch grains and the cell wall, concluded that the cell wall consists of ultramicroscopic, crystalline, molecular complexes which he called micellae. By this assumption he was able to explain striation, stratification, swelling, double refraction, and other properties of the cell wall." Subsequent workers have followed this general idea. Bailey (1, 2) points out that the cellulose consists of chains of anhydrous glucose residues which tend to aggregate in a parallel fashion. He also states that the aggregation of chain molecules is not in separate groups but rather a part of a continuous system which is held together by overlapping chain molecules and perforated by intercommunicating spaces. It is probably in these intercommunicating spaces that water is adsorbed and causes the changes in the dimension of wood. The mineral content is also probably localized here but

evidence of a continuous bond between inorganic material of the cell wall has not been found.

Conclusions

1. Silica in the form of continuous siliceous skeletons is not present in the secondary wall of woody tissue.

2. The silica content is such a small percentage of the total weight of wood that it could not have an appreciable effect, greater than other minerals, on the differential swelling or shrinking of wood.

3. Other than bringing about more rapid degradation of the wood substance, hydrofluoric acid is similar to hydrochloric acid in its action on the cell wall.

University of California, Berkeley,
May 28, 1942.

Literature Cited

1. Bailey, I. W. The cell and protoplasm. Publ. Amer. Assoc. Advancement Sci. no. 14: 31–43. 1940.
2. ————————. The microfibrillar and microcapillary structure of the cell wall. Bull. Torr. Bot. Club 66: 201–213. 1939.
3. Brown, F. B. H. The siliceous skeleton of tracheids and fibers. Bull. Torr. Bot. Club 47: 407–424. 1920.
4. ————————. The refraction of light in plant tissues. Bull. Torr. Bot. Club 47: 243–260. 1920.
5. Chamberlain, C. J. Methods in plant histology. Univ. of Chicago Press. 1938.
6. Forsaith, C. C. The technology of New York State timbers. Tech. Publ. 18. N. Y. State Coll. Forestry. 1926.
7. Hawley, L. F., and L. E. Wise. The chemistry of wood. The Chemical Catalog Co., Inc., New York. 1926.
8. Harlow, W. H. Contributions to the chemistry of the plant cell wall. IV. Tech. Publ. 26. N. Y. State Coll. Forestry. 1928.
9. Hillebrand, W. F., and G. E. F. Lundell. Applied inorganic analysis. John Wiley and Sons, New York.
10. Kerr, I. The action of hydrofluoric acid in softening. Tropical Woods 40: 37–41. 1934.
11. Kerr, I., and I. W. Bailey. The visible structure of the secondary wall. . . . Journ. Arn. Arb. 16: 273–300. 1935.
12. Maby. J. C. Micellar structure of the tracheid wall in certain woods in relation to morphogenetic and mechanical factors. New Phyt. 35: 432–455. 1936.
13. Nägeli, C. W. Die Anwendung des Polarisationsmikroskops auf die Untersuchung der organischen Elementartheile. Beitr. Wiss. Botan. 3: 1–126. 1863.
14. Plowman, A. B. The celloiden method for hard tissues. Bot. Gaz. 37: 456–461. 1904.
15. Record, S. J. Timbers of tropical America. Yale Univ. Press. New Haven, Conn. 1924.
16. Rudiger, W. Papierfabrickant. 38: 9–13. 1940.
17. Sacc, J. E. Elements of botanical microtechnique. McGraw-Hill. New York. 1940.
18. Schorger, A. W. The chemistry of cellulose and wood. McGraw-Hill. New York. 1926.
19. Uber, F. M. Microincineration and ash analysis. Bot. Review 6: 204–226. 1940.